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RESEARCH ARTICLE

A dynamic nomogram for predicting unfavorable prognosis after aneurysmal subarachnoid hemorrhage

Dongzhou Zhuang[1,*], Zhihui Ren[2,*], Jiangtao Sheng[2], Zenan Zheng[3], Hui Peng[4], Xurong Ou[5], Yuan Zhong[5], Tian Li[2], Shousen Wang[1], Kangsheng Li[2] & Weiqiang Chen[5]

[1]Department of Neurosurgery, Fuzong Clinical College of Fujian Medical University, 900 Hospital, Fuzhou, China
[2]Department of Microbiology and Immunology & Key Immunopathology Laboratory of Guangdong Province, Shantou University Medical College, Shantou, China
[3]Department of Cardiovascular Medicine, Shantou University Medical College, Shantou, China
[4]Department of Neurosurgery, Affiliated Jieyang People's Hospital of Sun Yat-sen University, Jieyang, China
[5]Department of Neurosurgery, First Affiliated Hospital, Shantou University Medical College, Shantou, China

Correspondence
Shousen Wang, Department of Neurosurgery, 900TH Hospital of Joint Logistics Support Force, Fuzong Clinical College of Fujian Medical University, Fuzhou 350025, China. Tel: +86-0591-24937080; Fax: +86-0591-87640785; E-mail: wshsen@xmu.edu.cn

Kangsheng Li, Department of Microbiology and Immunology & Key Immunopathology Laboratory of Guangdong Province, Shantou University Medical College, 22 Xinling Road, Shantou, Guangdong 515000, China. Tel: +86-0754-88900840; Fax: +86-0754-88557562; E-mail: ksli@stu.edu.cn

Weiqiang Chen, Department of Neurosurgery, First Affiliated Hospital, Shantou University Medical College, 57 Changping Road, Shantou, Guangdong 515041, China. Tel: +86-0754-88905326; Fax: +86-0754-88259850; E-mail: wqchen@stu.edu.cn

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*Dongzhou Zhuang and Zhihui Ren contributed equally to this work and share the first authorship.

Abstract

Objective: The aim of this study was to examine the predictive value of the multiplication of neutrophil and monocyte counts (MNM) in peripheral blood, and develop a new predictive model for the prognosis of patients with aneurysmal subarachnoid hemorrhage (aSAH). **Methods**: This is a retrospective analysis that included 2 separate cohorts of patients undergoing endovascular coiling for aSAH. The training cohort consisted of 687 patients in the First Affiliated Hospital of Shantou University Medical College; the validation cohort consisted of 299 patients from Sun Yat-sen University's Affiliated Jieyang People's Hospital. The training cohort was used to develop 2 models to predict unfavorable prognosis (modified Rankin scale of 3–6 at 3 months): one was based on traditional factors (e.g., age, modified Fisher grade, NIHSS score, and blood glucose), and another model that included traditional factors as well as MNM on admission. **Results**: In the training cohort, MNM upon admission was independently associated with unfavorable prognosis (odds ratio after adjustment, 1.06; 95% confidence interval [CI], 1.03–1.10). In the validation cohort, the basic model that included only traditional factors had 70.99% sensitivity, 84.36% specificity, and 0.859 (95% CI, 0.817–0.901) area under the receiver operating characteristic curve (AUC). Adding MNM increased model sensitivity (from 70.99% to 76.48%), specificity (from 84.36% to 88.63%), and overall performance (AUC 0.859 [95% CI, 0.817–0.901] to 0.879 [95% CI, 0.841–0.917]). **Interpretation**: MNM upon admission is associated with unfavorable prognosis in patients undergoing endovascular embolization for aSAH. The nomogram including MNM is a user-friendly tool to help clinicians quickly predict the outcome of patients with aSAH.

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a potentially fatal disease.[1] Re-bleeding can be prevented by open surgical clipping or endovascular embolization, but at least one-third of patients do not survive, and at least 20% of survivors never achieve functional independence.[2] Growing evidence shows that inflammation can influence

outcomes in patients with aSAH,[3] as well as intracerebral hemorrhage and ischemic stroke.[4,5] In aSAH, inflammatory damage is a critical contributor to cerebral vasospasm (CVS) and delayed cerebral ischemia.[6] This indicates that inflammation plays an important role in the development of aSAH and affects the prognosis of patients.[7]

Currently, reported inflammatory indicators of outcomes after aSAH include leukocyte count,[8–11] monocyte count,[12,13] neutrophil-to-lymphocyte ratio (NLR),[1,14,15] monocyte-to-lymphocyte ratio (MLR),[16,17] and C-reactive protein.[18,19] The NLR and MLR reflect the balance of change between innate and adaptive immunity and offer a simple indicator of immune status and inflammation level. aSAH patients with poor prognoses have elevated neutrophils, monocytes, and decreased lymphocytes. As an inexpensive and readily available biomarker, NLR and MLR recently have been suggested to predict prognosis in various diseases. Similarly, the MNM as a composite indicator of inflammation theoretically should be associated with the development and prognosis of patients with aSAH.

However, only a few studies have also shown that elevated MNM is associated with prognosis, and has involved either epithelial ovarian cancer or detection of early cervical cancer,[20,21] and the value of MNM has not been explored in the field of neuroscience. After aSAH, both clinical and animal experimental studies have associated elevated neutrophil and monocyte counts with poor prognosis.[12,22] Theoretically, as an inflammatory indicator, MNM is the product of elevated neutrophils and monocytes and should amplify the prognostic capability of both elevations. Therefore, we conducted a retrospective analysis to examine the potential relationship between MNM in the early course of aSAH and patient prognosis.

Patients and Methods

Patient population

This study consisted of 2 independent cohorts: patients treated with endovascular coil embolization for spontaneous aSAH at the First Affiliated Hospital of Shantou University Medical College from January 1, 2014, to September 30, 2019 (training cohort) and at Sun Yat-sen University's Affiliated Jieyang People's Hospital from December 18, 2019, to May 1, 2021 (external validation cohort). The flow chart of patient selection shows the inclusion and exclusion criteria for selecting patients with aSAH for this study (Fig. 1). The study was approved by the Ethics Committees of both centers (B-2021-244 and 2021097, respectively), and conducted in accordance with the ethical standards set out in the Declaration of Helsinki (as amended in 2013). The diagnosis of aSAH was established by CT at admission. The diagnosis of the intracranial aneurysm was established by CT angiography (CTA), magnetic resonance angiography, or digital subtraction angiography (DSA). We usually diagnose hydrocephalus by CT. Hydrocephalus is diagnosed when the temporal horn (TH) diameter is greater than 2 mm along with the absence of Sylvian and interhemispheric cistern, and the absence of cerebral sulci or the TH width is greater than 2 mm and the frontal horn (FH) diameter to internal diameter (ID) is greater than 0.5 (where the FH is the largest width of the frontal horns and the ID is the internal diameter from inner-table to inner-table at this level). Acute hydrocephalus is usually treated with external ventricular drainage, while chronic hydrocephalus is usually treated with a ventriculoperitoneal shunt.[23] Patients with other types of vascular malformation were excluded. All patients with aSAH received standard-of-care management according to the American Stroke Association guidelines.[24]

Definition of follow-up and outcome

The modified Rankin Scale (mRS) score was used to assess patients at the 3-month follow-up.[1,25] The mRS scores were as described previously: 0, no symptoms at all; 1, no obvious functional impairment despite symptoms, and the patient was able to perform all daily duties and activities; 2, mild disability, where the patient was able to take care of themselves without assistance but unable to complete all pre-disease activities; 3, moderate disability, where the patient was able to walk without assistance; 4, severe disability, where the patient was unable to walk without assistance and unable to care for their own physical needs; 5, the patient was bedridden; and 6, death. An unfavorable prognosis was defined as an mRS of 3–6. Follow-up of all patients was assessed jointly by two trained health managers in the clinic, in the hospital, or via telephone interview. Patient data were entered simultaneously into the follow-up system available to clinicians.

Statistical analysis

Statistical analyses were performed using Statistical Product and Service Solutions (26th version; IBM Corporation, Armonk, New York, USA) and R (version 4.1.0; R Foundation, Vienna, Austria).[26–29] Continuous variables are shown as the median and interquartile range (IQR); categorical variables are shown as count and percentage.

We further applied a two-piecewise linear regression model, using a smoothing function, to examine the

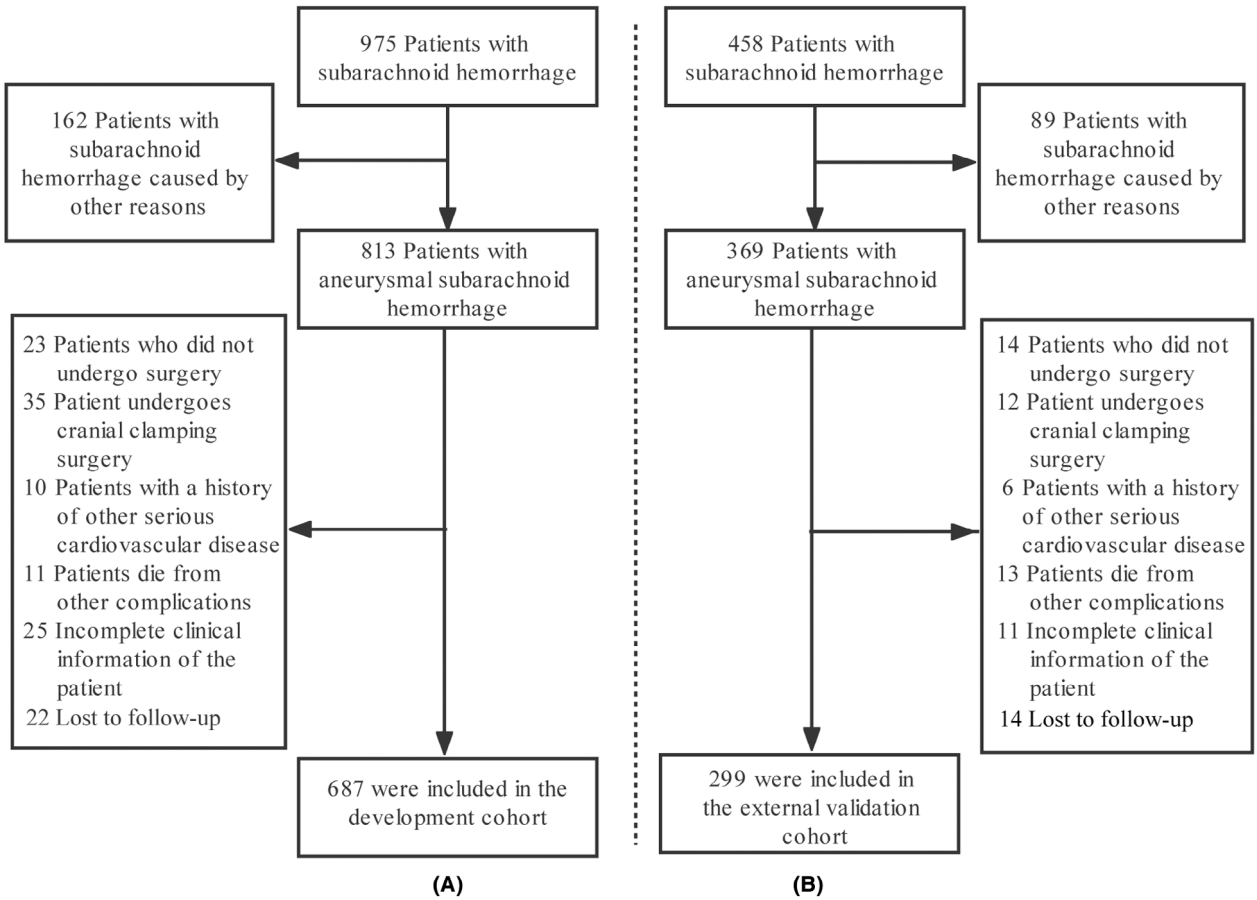

Figure 1. Flow chart of the patient selection process, including inclusion and exclusion criteria. (A) Patients in the training cohort were selected retrospectively from the First Affiliated Hospital of Shantou University Medical College from January 1, 2014, to September 30, 2019. (B) Patients in the validation cohort were prospectively selected from Sun Yat-sen University's Affiliated Jieyang People's Hospital from December 18, 2019, to May 1, 2021.

threshold effect of the MNM on unfavorable prognosis.[30] This function can intuitively show the change in the probability of poor prognosis with the change of MNM, and more directly show the relationship between MNM and prognosis. The interaction between MNM and relevant factors on unfavorable prognosis was also examined. A multivariate regression analysis with a forward selection procedure was conducted to identify independent factors that were associated with unfavorable prognoses. All parameters showing a statistical trend ($p < 0.1$) in univariate analysis were included in a multivariate regression model to identify parameters that were independently associated with unfavorable outcomes at 3 months. Also, commonly used or reported indicators, such as age, sex, smoking, alcohol consumption, blood pressure, glucose, and hyperlipidemia, were included in the multivariate regression analysis. Independent risk factors of unfavorable outcomes with the forward selection procedure, retaining variables with $p < 0.1$, were selected for multivariate regression analysis. The results of the regression analysis are shown as odds ratio (OR) and 95% confidence interval (95%CI). Two separate models were used: one based on already established independent risk factors (e.g., age, modified Fisher grade, NIHSS score, and blood glucose), and another that included the already established independent risk factors plus MNM. The area under the ROC curve was used to judge the predictive value of the models.[31] Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to compare the predictive power of the two models. Decision curve analysis (DCA) was performed to evaluate the clinical value of the models.[32,33] Finally, a nomogram was constructed based on the coefficient for each factor in the regression analysis. The predictive effect of the nomogram was verified using a graph calibration method, as previously described.[34]

Table 1. Differences between with favorable prognosis group and the unfavorable prognosis group in the development cohort (univariate analysis).

Variable	Total (n = 687)	Unfavorable prognosis (n = 264)	Favorable prognosis (n = 423)	p-value
Gender (male)	265 (38.57%)	103 (39.02%)	162 (38.30%)	0.915
Age	58 (50–65)	60 (52–67)	56 (48–63)	<0.001
Smoking	44 (6.40%)	10 (3.79%)	34 (8.04%)	0.031
Alcohol abuse	34 (4.95%)	7 (2.65%)	27 (6.38%)	0.044
Hypertension	270 (39.30%)	117 (44.32%)	153 (36.17%)	0.041
Diabetes	49 (7.13%)	25 (9.47%)	24 (5.67%)	0.084
Dyslipidemia (Yes vs No)	99 (14.41%)	32 (12.1%)	67 (15.8%)	0.216
History of stroke (Yes vs No)	3 (0.44%)	3 (1.14%)	0 (0%)	0.056
Level on GCS score				<0.001
Mild (13–15 score)	455 (66.23%)	98 (37.12%)	357 (84.40%)	
Moderate (9–12 score)	94 (13.68%)	52 (19.70%)	42 (9.93%)	
Severe (3–8 score)	138 (20.09%)	114 (43.18%)	24 (5.67%)	
Hunt-Hess grade				<0.001
I	137 (19.94%)	27 (10.23%)	110 (26.00%)	
II	131 (19.07%)	21 (7.95%)	110 (26.00%)	
III	266 (38.72%)	92 (34.85%)	174 (41.13%)	
IV	130 (18.92%)	102 (38.64%)	28 (6.62%)	
V	23 (3.35%)	22 (8.33%)	1 (0.24%)	
mFS grade				<0.001
0	3 (0.44%)	3 (1.14%)	0 (0%)	
I	107 (15.57%)	16 (6.06%)	91 (21.51%)	
II	347 (50.51%)	111 (42.05%)	236 (55.79%)	
III	138 (20.09%)	90 (34.09%)	48 (11.35%)	
IV	92 (13.39%)	44 (16.67%)	48 (11.35%)	
NIHSS score	2 (0–13)	17.5 (3–36)	1 (0–4)	<0.001
Cerebral parenchymal hematoma (Yes vs No)	112 (16.30%)	70 (26.52%)	42 (9.93%)	<0.001
Hydrocephalus (Yes vs No)	50 (7.29%)	35 (13.26%)	15 (3.55%)	<0.001
Location of aneurysm				0.887
Internal carotid artery	119 (17.32%)	46 (17.42%)	73 (17.26%)	
Middle cerebral artery	185 (26.93%)	77 (29.17%)	108 (25.53%)	
Former traffic artery	239 (34.79%)	89 (33.71%)	150 (35.46%)	
Rear traffic artery	122 (17.76%)	45 (17.05%)	77 (18.20%)	
Vertebral artery	14 (2.04%)	5 (1.89%)	9 (2.13%)	
Basilar artery	8 (1.16%)	2 (0.76%)	6 (1.42%)	
Multiple (Yes vs No)	96 (13.97%)	39 (14.77%)	57 (13.48%)	0.716
Stent implantation (Yes vs No)	92 (13.39%)	33 (9.07%)	59 (13.95%)	0.669
Blood glucose	8.40 (7.00–10.79)	9.75 (8.07–12.38)	7.80 (6.66–9.40)	<0.001
MAP, mmHg	136 (120–153)	133.33 (118.50–149.83)	139.83 (122.67–156.33)	0.002
Serum calcium level	2.27 (2.18–2.36)	2.27 (2.18–2.36)	2.27 (2.17–2.36)	0.947
Leukocyte count	13.84 (10.67–17.01)	15.70 (12.61–20.08)	12.41 (9.64–15.62)	<0.001
Neutrophil count	11.61 (8.25–14.64)	13.32 (10.54–17.32)	10.09 (7.62–13.36)	<0.001
Lymphocyte count	1.36 (0.91–2.00)	1.38 (0.90–2.32)	1.35 (0.92–1.88)	0.148
Monocyte count	0.56 (0.38–0.78)	0.66 (0.44–0.94)	0.51 (0.35–0.70)	<0.001
Platelet count	236 (197–275)	242.00 (204.75–275.25)	231.00 (194.00–274.00)	0.046
NLR	8.81 (4.98–14.03)	10.74 (5.75–15.38)	8.16 (4.65–13.04)	<0.001
MLR	0.38 (0.26–0.58)	0.43 (0.28–0.66)	0.37 (0.25–0.53)	<0.001
PLR	169.52 (115.19–254.10)	167.76 (104.95–261.55)	169.77 (122.63–247.56)	0.594
NWR	0.85 (0.78–0.90)	0.86 (0.80–0.90)	0.85 (0.76–0.89)	0.012
MNM	5.70 (3.25–10.21)	8.19 (4.99–15.02)	4.67 (2.85–8.12)	<0.001

GCS, Glasgow Coma Scale; MAP, mean arterial pressure; mFS grade, modified Fisher grade; MLR, monocyte to lymphocyte ratio; MNM, multiplication to neutrophil and monocyte counts; NIHSS, National Institutes of Health Stroke Scale; NLR, neutrophil to lymphocyte ratio; NWR, neutrophil to leukocyte ratio; PLR, platelet to lymphocyte ratio.

Results

Characteristics of the patients in the training cohort

A total of 986 patients were included in the final analysis: 687 in the training cohort, and 299 in the validation cohort. Among the 687 patients in the training cohort, 264 (38.43%) had an unfavorable prognosis (mRS score of 3 or above at the 3-month follow-up). In comparison to the patients with favorable prognosis ($n = 423$), Patients with unfavorable prognosis were older, more likely to have a severe or moderate GCS score, higher NIHSS score, higher Hunt-Hess grade and mFS score, and higher blood glucose on admission (Table 1). Patients with unfavorable prognoses also had higher leukocyte count, lymphocyte count, monocyte count, NLR, MLR, and MNM.

MNM as a risk factor for unfavorable prognosis

Univariate regression using the training cohort revealed an association between higher MNM and unfavorable prognosis (OR, 1.10; 95%CI, 1.07–1.13; Table S1). Also, higher MNM correlated with higher mRS score (Fig. 2A). MNM did not interact with any variables, including age, GCS score, modified Fisher grade, NIHSS score, and Hunt–Hess grade in terms of impact on unfavorable prognosis (Fig. S1). In multivariate regression, higher MNM remained associated with unfavorable prognosis after adjustment for other independent risk factors (OR, 1.06; 95% CI, 1.03–1.10; Table 2). There was a non-linear positive correlation between MNM and the rate of unfavorable prognosis (Fig. 2B). The AUC of the MNM in predicting unfavorable prognosis was 0.707 (95% CI, 0.668–0.747; Fig. 3A). In contrast, an unfavorable prognosis was not predicted by either NLR (AUC = 0.577) or MLR (AUC = 0.578). Other indicators that predicted unfavorable prognosis included GCS score, mFS grade, Hunt-Hess grade, and NIHSS score (Fig. 3B).

Table 2. The multivariate regression analysis for predicting unfavorable prognosis.

Variable	Odds ratio (95% CI)	*p*-value
Age	1.04 (1.02, 1.06)	<0.001
mFS grade		0.001
0, I, II	1 [Reference]	1 [Reference]
III, IV	2.02 (1.34, 3.04)	<0.001
NIHSS score	1.08 (1.06, 1.10)	<0.001
Blood glucose	1.09 (1.01, 1.17)	0.026
MNM	1.06 (1.03, 1.10)	<0.001

mFS grade, modified Fisher grade; MNM, multiplication to neutrophil and monocyte counts; NIHSS, National Institutes of Health Stroke Scale.

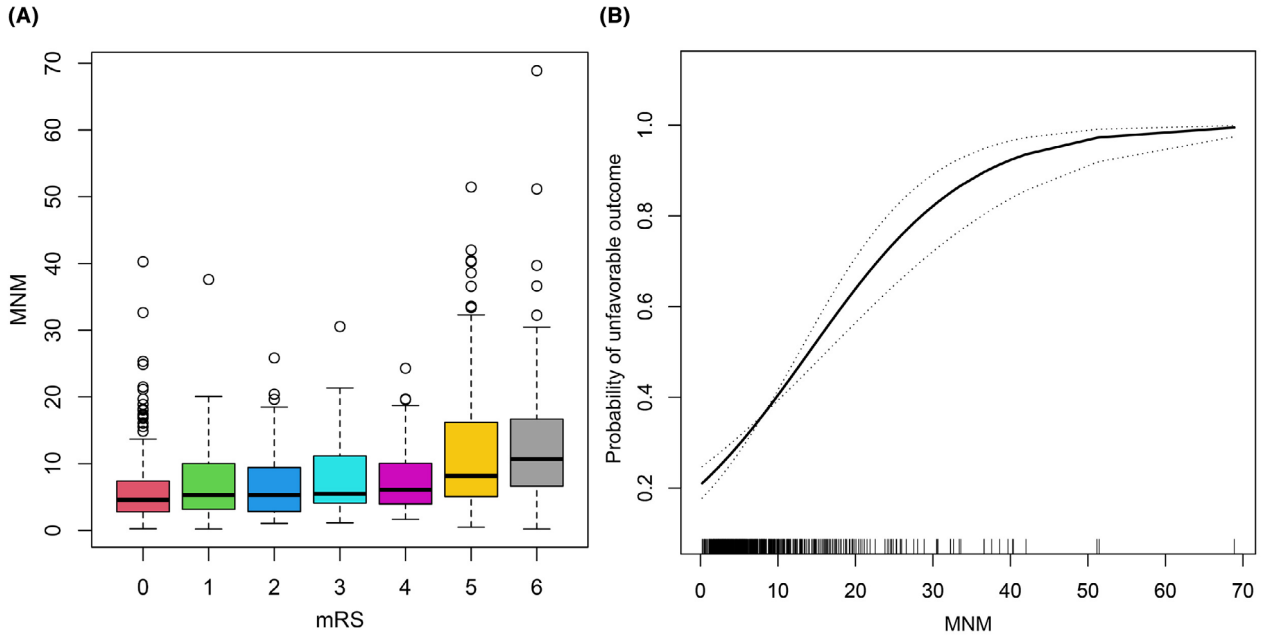

Figure 2. MNM level is positively associated with unfavorable prognosis in patients with aSAH. (A) The box diagram indicates that the distribution of MNM varies with the MRS score in different grades. (B) A non-linear relationship exists between the probability of unfavorable prognosis and the MNM level after aSAH.

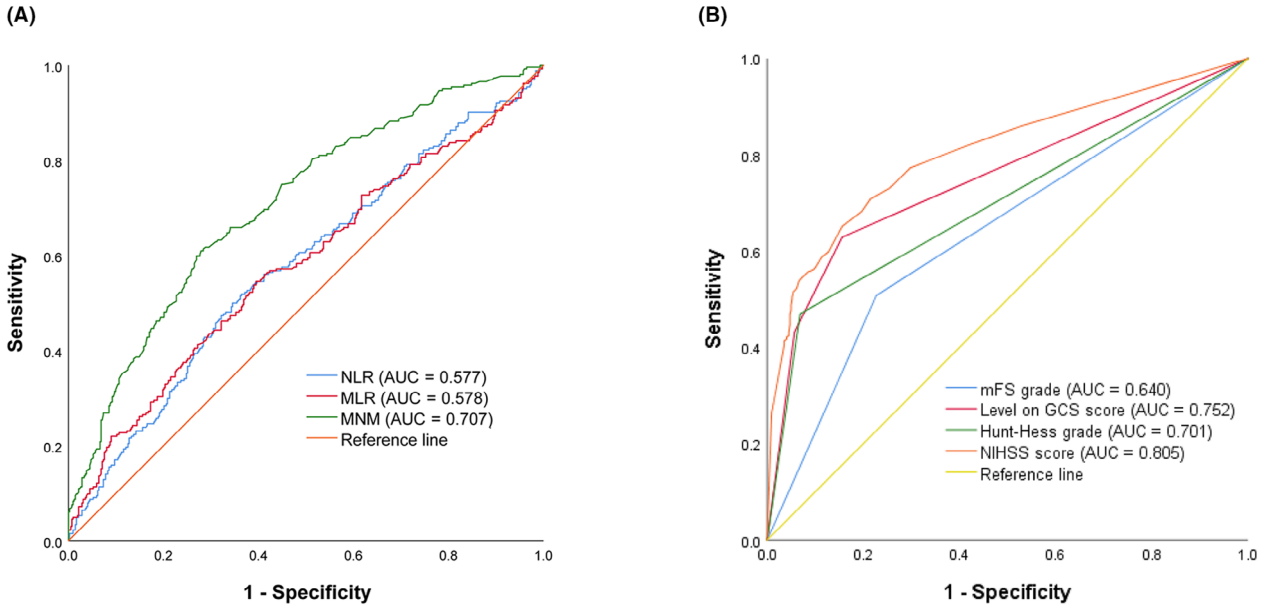

Figure 3. The area under the ROC curve of each index was compared. (A) ROC curve analysis of MNM, NLR, and MLR for unfavorable prognosis in the training cohort. (B) ROC curve analysis of other factors, such as mFS grade, GCS score, Hunt-Hess grade, and NIHSS score for unfavorable prognosis in the training cohort.

Predictive models for unfavorable prognosis

The predictive performance of the 2 models (i.e., with vs. without MNM) in the training cohort is shown in Table 2. In comparison to the basic model based on traditional factors (age, modified Fisher grade, NIHSS score, and blood glucose), adding MNM into the modeling (full model) did not significantly increase sensitivity (68.18% vs. 65.50%), but significantly increased specificity (90.31% vs. 83.45%) and the overall performance (AUC, 0.844 [95%CI, 0.816–0.878] vs. 0.827 [95%CI, 0.789–0.856]) (Fig. 4A). DCA suggested a net benefit of adding MNM into the model, with a threshold probability between 10% and 90% (Fig. 4B). Calibration curves suggested better agreement between prediction and observation in the full model than in the basic model (Fig. 4C,D). The corresponding median NRI and IDI values were 0.281 (95% CI 0.038–0.524; $p = 0.005$) and 0.030 (95% CI 0.008–0.051; $p = 0.007$), respectively (Fig. S2A).

A nomogram built on the full model for unfavorable prognosis was introduced to facilitate clinical application (Fig. 5). In the nomogram, the score includes the single item score (points in the figure), which represents the single item score corresponding to each variable with different values, and the total points represents combined the single item score corresponding to all variables. The total score for each patient corresponds to the probability of an unfavorable prognosis.

To facilitate the application of the prediction model, we upgraded the nomogram to a dynamic nomogram and uploaded it to the shinyapps.io platform https://dzzhuang.shinyapps.io/outcome/ to predict the prognosis of aSAH patients. Users can submit the 5 features to the corresponding text box of the web page for calculation through the computer or mobile phone (Fig. 6). After calculating the output of the sample, the results page will display the probability of a poor prognosis, the 95% confidence interval, and the parameters of the model.

External validation

The demographic and clinical characteristics of the basic clinical data of the 299 patients in the validation cohort are presented in Table 3. The basic model based solely on clinical variables (age, modified Fisher grade, NIHSS score, and blood glucose) had 70.99% sensitivity, 84.36% specificity, and 0.859 AUC under the ROC curve. Adding MNM into the full model improved sensitivity (70.99% to 76.48%), specificity (84.36% to 88.63%), and overall performance (AUC 0.859 [95% CI, 0.817–0.901] to 0.879 [95% CI, 0.841–0.917]; Fig 7A). Calibration curves demonstrated good agreement between prediction and observation (Fig. 7B). The corresponding median NRI and IDI values were 0.457 (95% CI 0.231–0.682; $p < 0.001$) and 0.055 (95% CI 0.026–0.084; $p < 0.001$), respectively (Fig. S2B). According to the nomogram in Figure 5, each

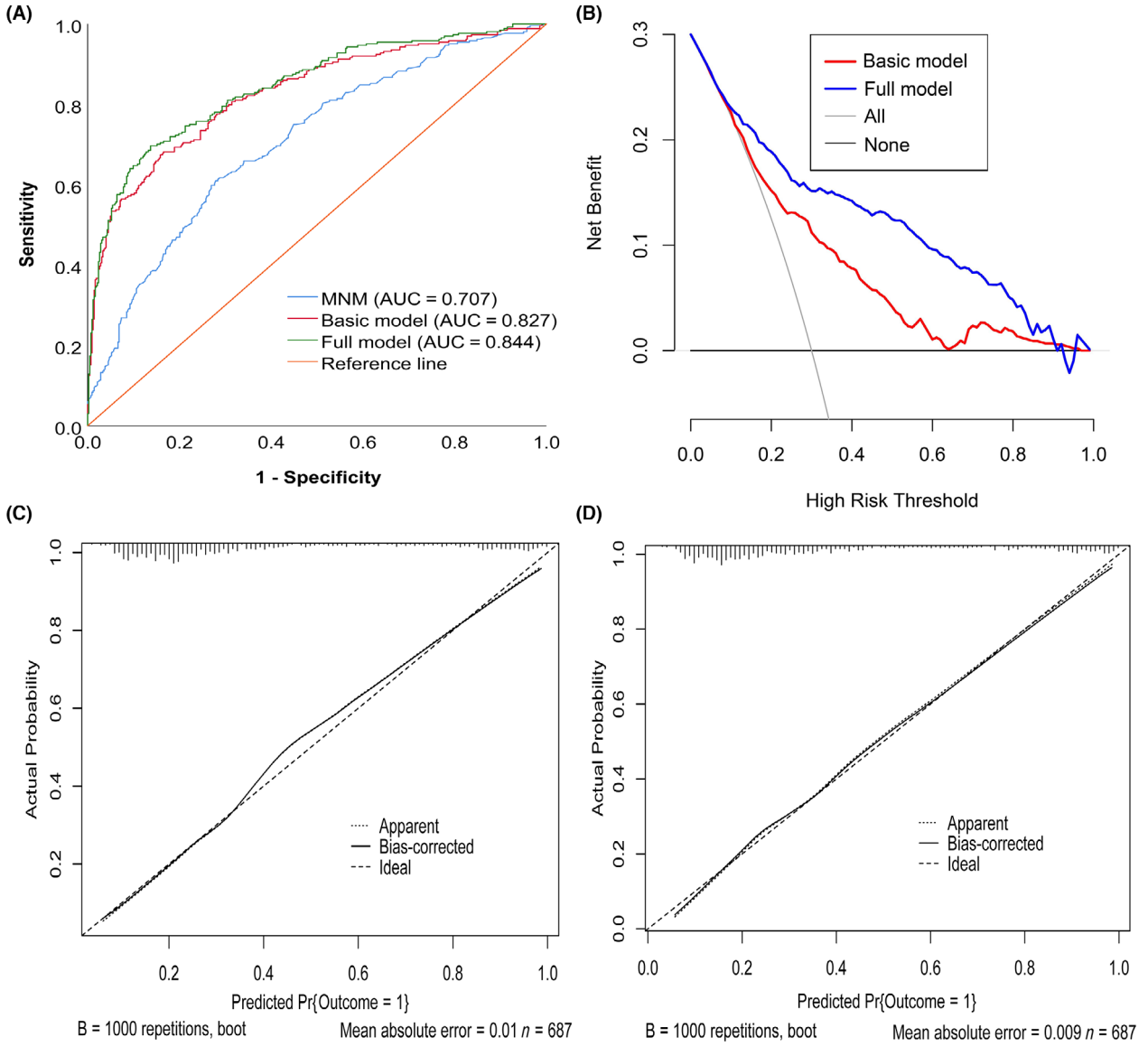

Figure 4. Establishment of the prediction model and demonstration of the prediction ability. (A) ROC curve analysis of MNM and basic and full models for unfavorable prognosis in the training cohort. (B) DCA curve of basic- and full model-predicted unfavorable prognosis. The full model shows a higher net benefit. (C) Calibration curves of the basic model predicted unfavorable prognosis in the validation cohort. (D) Calibration curves of the full model predicted unfavorable prognosis in the validation cohort.

patient receives a corresponding score, and the AUC of the corresponding score for predicting unfavorable prognosis was 0.837 (95% CI, 0.806–0.869; Fig. 7C).

Discussion

Results from the current study revealed an association between elevated MNM at admission and unfavorable prognosis in patients with aSAH. In multivariate regression using the data from the training cohort, unfavorable prognosis was independently associated with higher MNM as well as traditional factors, for example, age, mFS grade, NIHSS score, and blood glucose, that are already known. Consistent with previous studies,[10] older age was an independent predictor of prognosis (OR, 1.04; 95% CI, 1.02–1.06) in the current study. This suggests that older patients may have a worse prognosis due to lower immunity and more complications during hospitalization. The mFS grade is a radiologic tool that reflects the severity of subarachnoid hemorrhage and is known to be an independent risk factor for CVS.[35] The mFS grade has also been used to predict the outcomes of patients

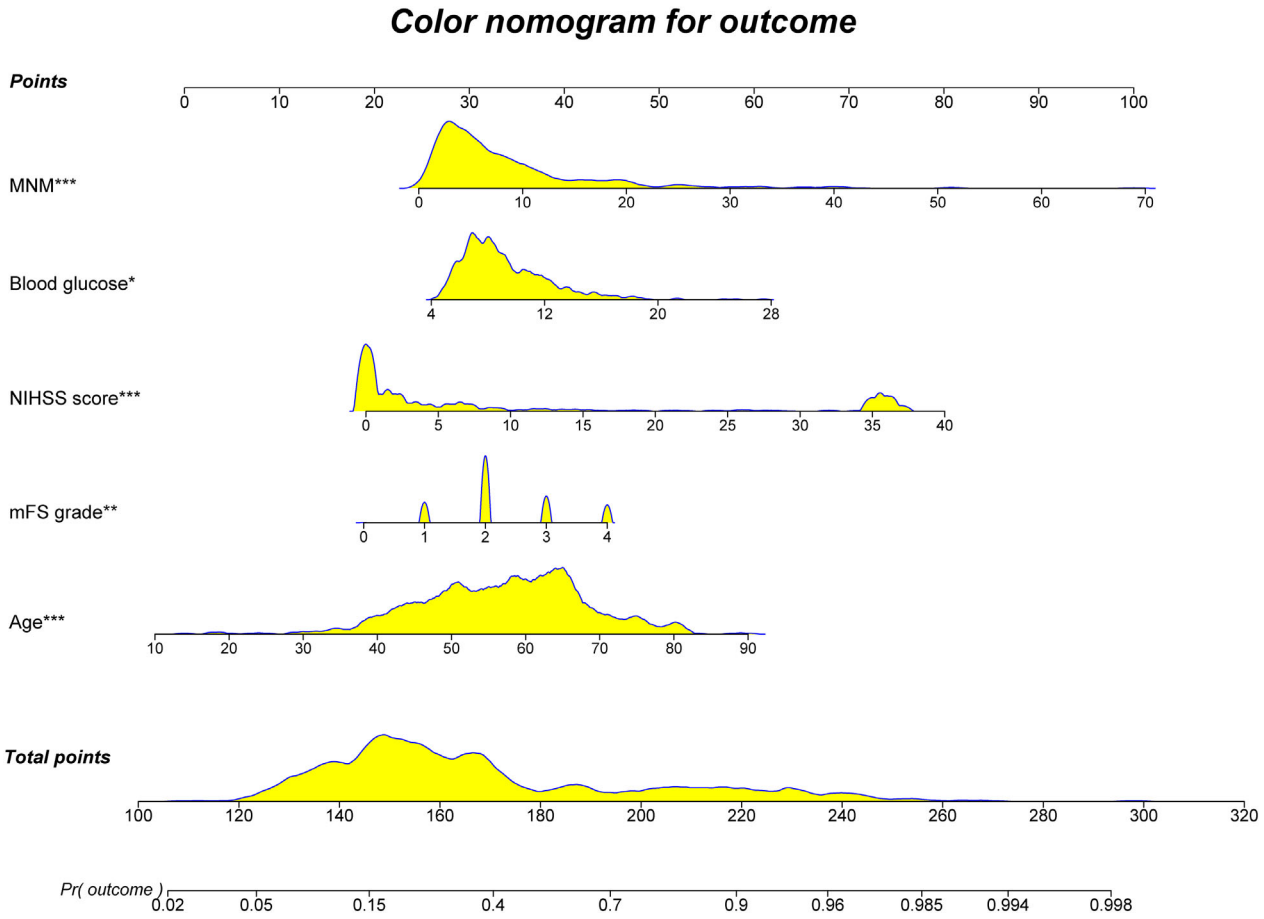

Figure 5. Nomogram, constructed from the full model, usage to predict unfavorable prognosis. Nomogram according to the various influencing factors on outcome variables in the model. The impact (the size of the regression coefficient for each level of each value of factors is assigned points, then the scores are added to get the total score, which determines the individual event prediction probability.

with aSAH.[1,34,36] In the current study, mFS grade was an independent predictor of unfavorable prognosis (OR, 2.02; 95% CI, 1.34–3.04; AUC, 0.64), confirming the study's validity. The NIHSS score reflects disease severity based on clinical symptoms, is particularly useful in assessing the severity of the ischemic stroke,[37] and is also an important risk factor for predicting unfavorable prognosis of aSAH patients.[38,39] The current findings also confirm a strong correlation between higher NIHSS scores and unfavorable prognosis of patients with aSAH (OR, 1.08; 95% CI, 1.06–1.10; AUC, 0.805). Also consistent with previous studies,[40–42] elevated blood glucose was associated with an unfavorable prognosis in the current study. Notably, hyperglycemia after the onset of aSAH was correlated with the original neurological symptoms and prognosis and may be attributed to metabolic changes caused by brain injury after aSAH.

Although incorporating MNM into clinical models only marginally improved the predictive performance of the development and validation cohorts, the DCA curves and calibration curves still suggested that MNM improved the accuracy and stability of the predictive models. Therefore, we cannot deny the value of MNM in prediction models. The introduction of MNM can always remind clinicians that inflammatory response plays an important role in the progression of aSAH patients and paves the way for subsequent studies. Moreover, MNM adds hematological indices to the prediction model, which along with basic clinical information, imaging indices, and clinical scores, increases the completeness of the model.

Previous literature suggests that NLR and MLR are associated with poor outcomes,[1,4,14] delayed cerebral ischemia,[43] and rebleeding.[15] In the present study, the unfavorable prognosis of patients with aSAH was more closely associated with increased MNM than with NLR and MLR. In our study, the AUC for NLR and MLR were 0.577 and 0.578, respectively, which appears to be inconsistent with previous studies. However, in the study by

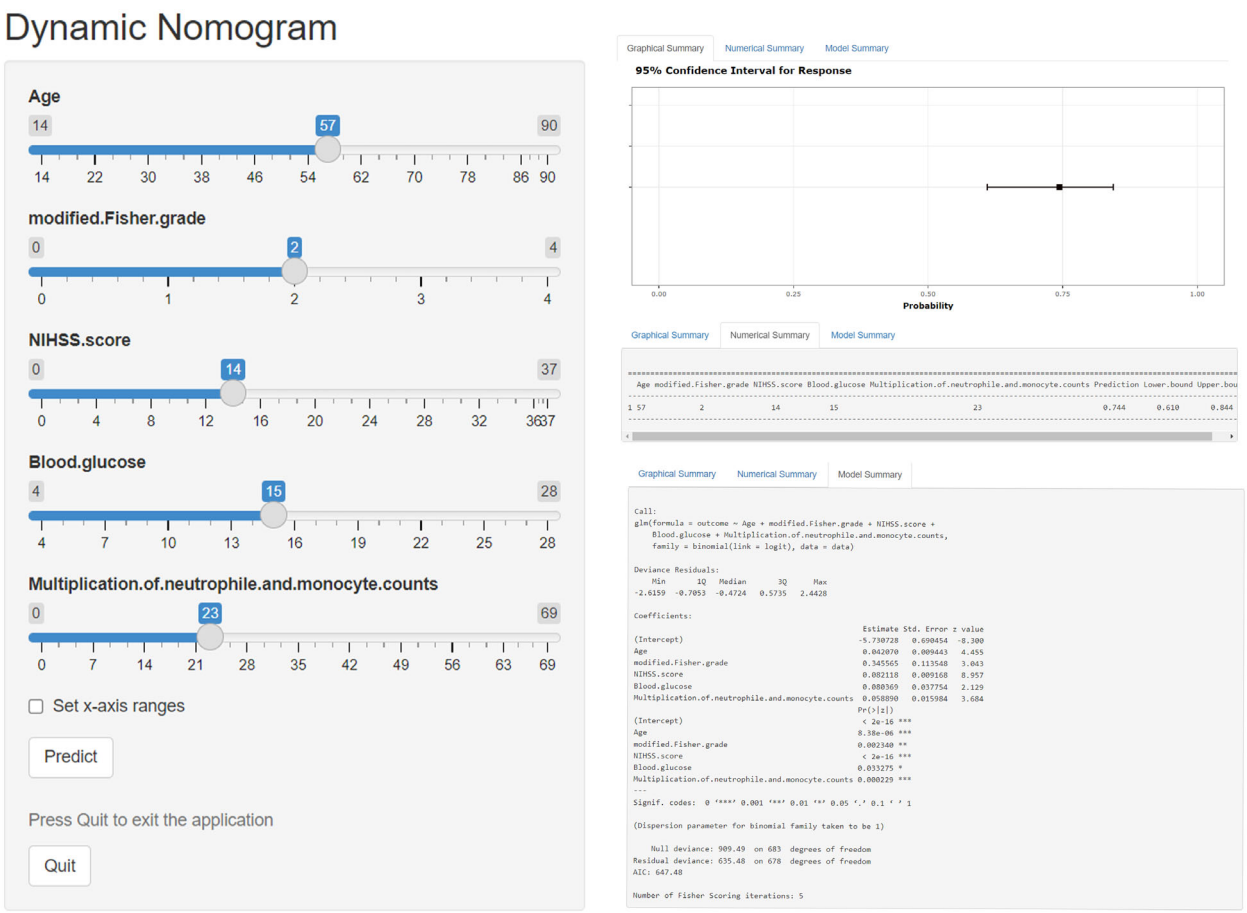

Figure 6. Shinyapps.io platform to predict the prognosis of patients with aSAH (https://dzzhuang.shinyapps.io/outcome/). Users can submit these 5 features to the corresponding text box of the web page through the computer or mobile phone for calculation. Once the output of the sample has been calculated, the results page will display the probability of a poor prognosis, the 95% confidence interval, and the parameters of the model.

Feghali et al.,[12] NLR and MLR also performed poorly in predicting the prognosis of patients with aSAH, with AUCs of 0.543 and 0.608 for NLR and MLR, respectively. Nevertheless, the article suggested that inflammatory indices that incorporate monocyte counts (e.g., M-NLR and MLR) are independently associated with poor long-term functional status and clinically significant vasospasm. Therefore, our study does not exclude the role of NLR and MLR in the prognosis of aSAH patients, which was also not the main purpose of the article.

Local and systemic inflammatory responses have a substantial influence on the prognosis of patients.[44] Upon aSAH, accumulated methemoglobin and heme in the subarachnoid space activate Toll-like receptor 4 to initiate an inflammatory cascade.[18,45,46] Blood in the subarachnoid space also activates immunoregulatory cells, such as microglia in the central nervous system.[45,46] Immunomodulatory cells promote the upregulation of cell adhesion molecules in endothelial cells, thus allowing a large number of inflammatory cells, such as neutrophils, monocytes, and macrophages, to infiltrate the subarachnoid space.[45,47,48] Neutrophils, the most common type of leukocyte in the peripheral circulation, are inflammatory cells that infiltrate the central nervous system most rapidly after peripheral immunization with aSAH.[19] In mouse models of aSAH, it takes only 10 minutes for neutrophils to migrate into the central nervous system.[49,50] Neutrophil infiltration increases the permeability of intercellular junctions between endothelial cells and facilitates the entry of other inflammatory cells to the site of injury.[51,52] Accumulated neutrophils may also block blood vessels and restrict blood flow to the brain.[53]

Monocyte activation is also an early response to tissue injury. Monocytes are involved in tissue repair and hemostasis during the acute phase and act as antigen-presenting cells that communicate with the immune

Table 3. Differences between with favorable prognosis group and the unfavorable prognosis group in the validation cohort (univariate analysis).

Variable	Total (n = 299)	Unfavorable prognosis (n = 117)	Favorable prognosis (n = 182)	p-value
Gender (male)	117 (39.13%)	40 (34.18%)	77 (42.31%)	0.200
Age	60 (51–66)	62 (55–68)	58 (49–65)	0.001
Smoking	22 (7.36%)	8 (6.84%)	14 (7.69%)	0.152
Alcohol abuse	22 (7.36%)	7 (5.98%)	15 (8.24%)	0.044
Hypertension	150 (50.17%)	66 (56.41%)	84 (46.15%)	0.107
Diabetes	19 (6.35%)	11 (9.40%)	8 (4.40%)	0.136
Dyslipidemia (Yes vs No)	42 (14.05%)	14 (11.97%)	28 (15.38%)	0.216
History of stroke (Yes vs No)	5 (1.67%)	4 (3.42%)	1 (0.55%)	0.046
Level on GCS score				<0.001
Mild (13–15 score)	143 (47.83%)	24 (20.51%)	119 (65.38%)	
Moderate (9–12 score)	72 (24.08%)	25 (21.37%)	47 (25.82%)	
Severe (3–8 score)	84 (28.09%)	68 (58.12%)	16 (8.79%)	
Hunt-Hess grade				<0.001
I	42 (14.05%)	5 (4.27%)	37 (20.33%)	
II	85 (28.43%)	13 (11.11%)	72 (39.56%)	
III	90 (30.10%)	30 (25.64%)	60 (32.97%)	
IV	63 (21.07%)	50 (42.74%)	13 (7.14%)	
V	19 (6.35%)	19 (16.24%)	0 (0%)	
mFS grade				<0.001
0	4 (1.34%)	1 (0.85%)	3 (1.65%)	
I	24 (8.03%)	1 (0.85%)	23 (12.64%)	
II	44 (14.72%)	8 (6.84%)	36 (19.78%)	
III	81 (27.09%)	62 (52.99%)	19 (10.44%)	
IV	146 (48.83%)	45 (38.46%)	101 (55.49%)	
NIHSS score	4 (1–13)	17.5 (3–36)	1 (0–4)	<0.001
Cerebral parenchymal hematoma (Yes vs No)	43 (14.38%)	28 (26.52%)	15 (9.93%)	<0.001
Hydrocephalus (Yes vs No)	21 (7.02%)	16 (13.26%)	5 (3.55%)	<0.001
Location of aneurysm				0.837
Internal carotid artery	51 (17.06%)	20 (17.09%)	31 (17.03%)	
Middle cerebral artery	80 (26.76%)	34 (29.06%)	46 (25.27%)	
Former traffic artery	104 (34.78%)	39 (33.33%)	65 (35.71%)	
Rear traffic artery	50 (16.72%)	19 (16.24%)	31 (17.03%)	
Vertebral artery	6 (2.06%)	3 (2.56%)	3 (1.65%)	
Basilar artery	8 (2.68%)	2 (1.71%)	6 (3.30%)	
Multiple (Yes vs No)	42 (14.05%)	17 (14.53%)	25 (13.74%)	0.616
Stent implantation (Yes vs No)	39 (13.04%)	10 (8.55%)	29 (15.93%)	0.627
Blood glucose	8.74 (7.30–10.57)	9.48 (7.60–11.77)	8.32 (7.07–9.85)	0.002
MAP, mmHg	111 (101–124)	115 (105–128)	109 (100–122)	0.019
Serum calcium level	2.17 (2.08–2.24)	2.16 (2.07–2.23)	2.17 (2.09–2.24)	0.250
Leukocyte count	13.50 (10.75–16.63)	15.79 (12.57–18.35)	12.44 (9.69–14.84)	<0.001
Neutrophil count	11.08 (7.96–14.01)	13.17 (8.57–16.77)	10.00 (6.12–13.88)	<0.001
Lymphocyte count	1.58 (0.98–2.28)	1.58 (1.05–2.48)	1.59 (0.94–2.19)	0.448
Monocyte count	0.64 (0.42–0.89)	0.77 (0.56–1.30)	0.52 (0.38–0.77)	<0.001
Platelet count	239.00 (201.00–289.00)	242.00 (212.00–295.00)	235.50 (193.50–287.00)	0.156
NLR	7.49 (3.77–12.06)	8.36 (4.48–13.32)	6.66 (3.38–11.14)	0.032
MLR	0.40 (0.25–0.64)	0.50 (0.30–0.88)	0.35 (0.23–0.50)	<0.001
PLR	152.35 (100.45–229.63)	150 (111.66–226.21)	152.41 (98.36–229.14)	0.779
NWR	0.82 (0.73–0.88)	0.83 (0.75–0.88)	0.82 (0.72–0.88)	0.309
MNM	6.72 (3.71–11.15)	10.09 (6.18–17.27)	5.14 (2.93–8.70)	<0.001

GCS, Glasgow Coma Scale; MAP, mean arterial pressure; mFS grade, modified Fisher grade; MLR, monocyte to lymphocyte ratio; MNM, multiplication to neutrophil and monocyte counts; NIHSS, National Institutes of Health Stroke Scale; NLR, neutrophil to lymphocyte ratio; NWR, neutrophil to leukocyte ratio; PLR, platelet to lymphocyte ratio.

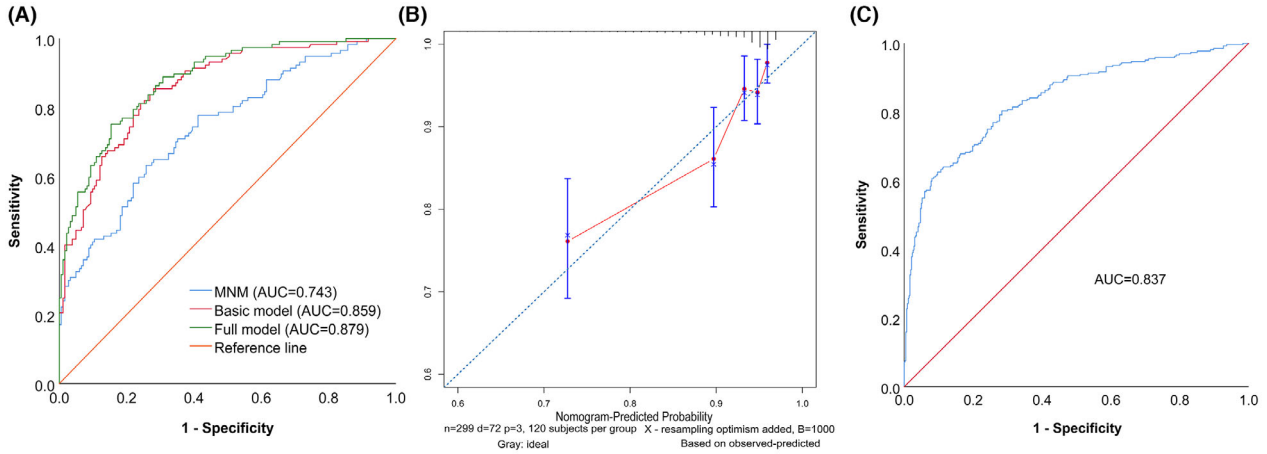

Figure 7. Validation of the predictive value of the prediction model. (A) ROC curve analyses of MNM and the basic (traditional factors only) and full (traditional factors plus MNM) models for unfavorable prognosis in the validation cohort. (B) Calibration curves of the nomogram prediction of unfavorable prognosis. (C) ROC curve analysis of the score of each patient from the nomogram for predicting unfavorable prognosis.

system.[10] In a preclinical mouse model, the brain after aSAH is infiltrated by an increased number of neutrophils, as well as monocytes, and shows an early reduction in non-classical monocytes. Monocytes proliferate rapidly after an aneurysm, enter brain tissue, and transform into a pro-inflammatory phenotype during early aSAH.[10,54] The mechanism of how neutrophils and monocytes induce neuroinflammation is complex. Studies have shown that aSAH also induces microglial activation.[52] Hyperactivated microglia can recruit monocytes to differentiate into brain macrophages by secreting numerous pro-inflammatory factors (i.e., tumor necrosis factor-alpha, interleukin-1β, and interleukin-6).[55] Finally, the early activation of microglia and astrocytes causes macrophages to be recruited and activated by neutrophils and monocytes, which cross the blood–brain barrier into the subarachnoid space during the early stages of aSAH and activate and maintain local cellular inflammation.[56]

Although we have tried to make this study complete and meaningful, there are still several limitations. First, patients treated conservatively and craniotomy for aneurysmal clamping were excluded from this study. Conservatively treated patients have a risk of re-rupture and bleeding of the aneurysm, and there are large differences in patient management and treatment protocols compared with surgical treatment, which can interfere greatly with disease progression and prognosis. As for the exclusion of patients with craniotomy for aneurysmal clamping, the purpose was also for unifying the surgical approach in order to avoid interference, by other treatment methods, of the surgical approach on prognosis, although the performance of the prediction model was not affected by the inclusion of these cases (AUC = 0.846,0.814–0.879). However, the number of cases of craniotomy for aneurysmal clamping was small. In 35 patients, the probability of poor prognosis and mortality was much higher than in patients treated endovascularly (73.7% vs 38.43%, and 31.6% vs 10.5%). Therefore, although the exclusion of this part of the data is a drawback of this study, it is indeed reasonable. Second, as a retrospective study, the results are subject to selection biases and missing information. The use of two independent cohorts may help to reduce the bias, but future prospective multicenter cohort studies are needed to validate the predictive value of MNM for unfavorable prognosis. Next, other inflammatory markers, such as C-reactive protein and interleukin-6, were not included in this study. Finally, although this study included Hunt–Hess grade, mFS grade, and GCS score, the World Federation of Neurosurgical Societies scores, as an important indicator of aSAH, were missing from our data.

Conclusions

Higher MNM at admission was associated with a 3-month unfavorable prognosis (mRS of 3 or higher) in patients with aSAH. The nomogram including MNM is a user-friendly tool to help clinicians quickly predict the outcome of patients with aSAH.

Author Contributions

Dongzhou Zhuang, Zhihui Ren, Weiqiang Chen, Kangsheng Li, and Shousen Wang were responsible for the study concept and design. Dongzhou Zhuang, Zhihui Ren, Jiangtao Sheng, and Zenan Zheng were responsible for the

analysis and interpretation of data. Zhihui Ren, Dongzhou Zhuang, Jiangtao Sheng, and WC were responsible for the drafting of the manuscript. Hui Peng, Zenan Zheng, Yuan Zhong, and Tian Li were responsible for data collection. Zhihui Ren, Dongzhou Zhuang, Weiqiang Chen, Kangsheng Li, and Shousen Wang provided critical intellectual contributions and participated in manuscript revision.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Statement

The study was approved by the Ethics Committees of the First Affiliated Hospital of Shantou University Medical College (B-2021-244) and Jieyang People's Hospital (2021097).

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Data Availability Statement

The datasets generated during and/or analyzed are available from the corresponding authors upon request.

References

1. Giede-Jeppe A, Reichl J, Sprügel MI, et al. Neutrophil-to-lymphocyte ratio as an independent predictor for unfavorable functional outcome in aneurysmal subarachnoid hemorrhage. J Neurosurg. 2019;132:400-407.
2. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. Stroke. 2010;41:e519-e536.
3. Sarrafzadeh A, Schlenk F, Meisel A, Dreier J, Vajkoczy P, Meisel C. Immunodepression after aneurysmal subarachnoid hemorrhage. Stroke. 2011;42:53-58.
4. Bhat T, Teli S, Rijal J, et al. Neutrophil to lymphocyte ratio and cardiovascular diseases: a review. Expert Rev Cardiovasc Ther. 2013;11:55-59.
5. Wang F, Hu S, Ding Y, et al. Neutrophil-to-lymphocyte ratio and 30-day mortality in patients with acute intracerebral hemorrhage. J Stroke Cerebrovasc Dis. 2016;25:182-187.
6. Zhang Z, Fang Y, Lenahan C, Chen S. The role of immune inflammation in aneurysmal subarachnoid hemorrhage. Exp Neurol. 2021;336:113535.
7. Pradilla G, Chaichana KL, Hoang S, Huang J, Tamargo RJ. Inflammation and cerebral vasospasm after subarachnoid hemorrhage. Neurosurg Clin N Am. 2010;21:365-379.
8. Neil-Dwyer G, Cruickshank J. The blood leucocyte count and its prognostic significance in subarachnoid Haemorrhage. Brain. 1974;97:79-86.
9. Al-Mufti F, Misiolek KA, Roh D, et al. White blood cell count improves prediction of delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. Neurosurgery. 2019;84:397-403.
10. McGirt MJ, Mavropoulos JC, McGirt LY, et al. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg. 2003;98:1222-1226.
11. Bacigaluppi S, Ivaldi F, Bragazzi NL, et al. An early increase of blood leukocyte subsets in aneurysmal subarachnoid hemorrhage is predictive of vasospasm. Front Neurol. 2020;11:587039.
12. Feghali J, Kim J, Gami A, et al. Monocyte-based inflammatory indices predict outcomes following aneurysmal subarachnoid hemorrhage. Neurosurg Rev. 2021;44:3499-3507.
13. Unda SR, Birnbaum J, Labagnara K, Wong M, Vaishnav DP, Altschul DJ. Peripheral monocytosis at admission to predict cerebral infarct and poor functional outcomes in subarachnoid hemorrhage patients. World Neurosurg. 2020;138:e523-e529.
14. Jamali SA, Turnbull MT, Kanekiyo T, et al. Elevated neutrophil-lymphocyte ratio is predictive of poor outcomes following aneurysmal subarachnoid hemorrhage. J Stroke Cerebrovasc Dis. 2020;29:104631.
15. Wang JY, Zhang XT, Wang JQ, et al. Admission neutrophil-lymphocyte ratio predicts rebleeding following aneurismal subarachnoid hemorrhage. World Neurosurg. 2020;138:e317-e322.
16. Park MG, Kim MK, Chae SH, Kim HK, Han J, Park KP. Lymphocyte-to-monocyte ratio on day 7 is associated with outcomes in acute ischemic stroke. Neurol Sci. 2018;39:243-249.
17. Song Q, Pan R, Jin Y, et al. Lymphocyte-to-monocyte ratio and risk of hemorrhagic transformation in patients with acute ischemic stroke. Neurol Sci. 2020;41:2511-2520.
18. Rothoerl RD, Axmann C, Pina AL, Woertgen C, Brawanski A. Possible role of the C-reactive protein and

white blood cell count in the pathogenesis of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2006;18:68-72.
19. Srinivasan A, Aggarwal A, Gaudihalli S, et al. Impact of early leukocytosis and elevated high-sensitivity C-reactive protein on delayed cerebral ischemia and neurologic outcome after subarachnoid hemorrhage. World Neurosurg. 2016;90:91-95.
20. Cho H, Kim JH. Multiplication of neutrophil and monocyte counts (MNM) as an easily obtainable tumour marker for cervical cancer. Biomarkers. 2009;14:161-170.
21. Paik ES, Shim M, Choi HJ, et al. Preoperative multiplication of neutrophil and monocyte counts as a prognostic factor in epithelial ovarian cancer. Cancer Biomark. 2016;17:419-425.
22. Neulen A, Pantel T, Kosterhon M, et al. Neutrophils mediate early cerebral cortical hypoperfusion in a murine model of subarachnoid haemorrhage. Sci Rep. 2019;9:8460.
23. Bhattacharjee S, Rakesh D, Ramnadha R, Manas P. Subarachnoid hemorrhage and hydrocephalus. Neurol India. 2021;69(Supplement):S429-S433.
24. Connolly ES Jr, Rabinstein AA, Carhuapoma JR, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2012;43(6):1711-1737.
25. Gathier CS, van den Bergh WM, van der Jagt M, et al. Induced hypertension for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: a randomized clinical trial. Stroke. 2018;49(1):76-83.
26. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate imputation by chained equations in R. J Stat Soft. 2010;45:1-68.
27. Null R, Team R, Null R, et al. R: a language and environment for statistical computing. Computing. 2011;1:12-21.
28. Sullivan LM, Massaro JM, D'Agostino RB Sr. Presentation of multivariate data for clinical use: the Framingham study risk score functions. Stat Med. 2004;23:1631-1660.
29. Sheng J, Li T, Zhuang D, et al. The monocyte-to-lymphocyte ratio at hospital admission is a novel predictor for acute traumatic intraparenchymal hemorrhage expansion after cerebral contusion. Mediators Inflamm. 2020;2020:5483981.
30. Yu X, Cao L, Yu X. Elevated cord serum manganese level is associated with a neonatal high ponderal index. Environ Res. 2013;121:79-83.
31. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology. 1982;143:29-36.
32. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006;26:565-574.
33. Lamain-de Ruiter M, Kwee A, Naaktgeboren CA, et al. External validation of prognostic models to predict risk of gestational diabetes mellitus in one Dutch cohort: prospective multicentre cohort study. BMJ. 2016;354:i4338.
34. Liu H, Xu Q, Li A. Nomogram for predicting delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage in the Chinese population. J Stroke Cerebrovasc Dis. 2020;29:105005.
35. Claassen J, Bernardini GL, Kreiter K, et al. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: the fisher scale revisited. Stroke. 2001;32:2012-2020.
36. Bae IS, Chun HJ, Choi KS, Yi HJ. Modified Glasgow coma scale for predicting outcome after subarachnoid hemorrhage surgery. Med (Baltim). 2021;100:e25815.
37. Kwah LK, Diong J. National Institutes of Health stroke scale (NIHSS). J Physiother. 2014;60:61.
38. Wang Y, Gao Y, Lu M, Liu Y. Long-term functional prognosis of patients with aneurysmal subarachnoid hemorrhage treated with rehabilitation combined with hyperbaric oxygen: case-series study. Med (Baltim). 2020;99:e18748.
39. Nylén K, Csajbok LZ, Ost M, et al. CSF-neurofilament correlates with outcome after aneurysmal subarachnoid hemorrhage. Neurosci Lett. 2006;404:132-136.
40. Kruyt ND, Roos YW, Dorhout Mees SM, et al. High mean fasting glucose levels independently predict poor outcome and delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry. 2008;79:1382-1385.
41. Kruyt ND, Biessels GJ, DeVries JH, et al. Hyperglycemia in aneurysmal subarachnoid hemorrhage: a potentially modifiable risk factor for poor outcome. J Cereb Blood Flow Metab. 2010;30:1577-1587.
42. Beseoglu K, Steiger HJ. Elevated glycated hemoglobin level and hyperglycemia after aneurysmal subarachnoid hemorrhage. Clin Neurol Neurosurg. 2017;163:128-132.
43. Cai L, Zeng H, Tan X, Wu X, Qian C, Chen G. The role of the blood neutrophil-to-lymphocyte ratio in aneurysmal subarachnoid hemorrhage. Front Neurol. 2021;12:671098.
44. Hokari M, Uchida K, Shimbo D, Gekka M, Asaoka K, Itamoto K. Acute systematic inflammatory response syndrome and serum biomarkers predict outcomes after subarachnoid hemorrhage. J Clin Neurosci. 2020;78:108-113.
45. Lucke-Wold BP, Logsdon AF, Manoranjan B, et al. Aneurysmal subarachnoid hemorrhage and neuroinflammation: a comprehensive review. Int J Mol Sci. 2016;17:497.
46. Ascenzi P, Bocedi A, Visca P, et al. Hemoglobin and heme scavenging. IUBMB Life. 2005;57:749-759.
47. Hailer NP, Bechmann I, Heizmann S, Nitsch R. Adhesion molecule expression on phagocytic microglial cells

following anterograde degeneration of perforant path axons. Hippocampus. 1997;7:341-349.
48. Gallia GL, Tamargo RJ. Leukocyte-endothelial cell interactions in chronic vasospasm after subarachnoid hemorrhage. Neurol Res. 2006;28:750-758.
49. Friedrich V, Flores R, Muller A, Bi W, Peerschke EI, Sehba FA. Reduction of neutrophil activity decreases early microvascular injury after subarachnoid haemorrhage. J Neuroinflammation. 2011;8:103.
50. Coulibaly AP, Provencio JJ. Aneurysmal subarachnoid hemorrhage: an overview of inflammation-induced cellular changes. Neurotherapeutics. 2020;17:436-445.
51. Forsyth KD, Simpson AC, Fitzpatrick MM, Barratt TM, Levinsky RJ. Neutrophil-mediated endothelial injury in haemolytic uraemic syndrome. Lancet. 1989;2:411-414.
52. Smedly LA, Tonnesen MG, Sandhaus RA, et al. Neutrophil-mediated injury to endothelial cells. Enhancement by endotoxin and essential role of neutrophil elastase. J Clin Invest. 1986;77:1233-1243.
53. Xue M, Del Bigio MR. Intracortical hemorrhage injury in rats: relationship between blood fractions and brain cell death. Stroke. 2000;31:1721-1727.
54. Gris T, Laplante P, Thebault P, et al. Innate immunity activation in the early brain injury period following subarachnoid hemorrhage. J Neuroinflammation. 2019;16:253.
55. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol. 2013;229:176-185.
56. Kubota T, Handa Y, Tsuchida A, Kaneko M, Kobayashi H, Kubota T. The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. Stroke. 1993;24:1993-2000.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. OR (95%CI) for prognosis in subgroups of aSAH patients and interaction test of the stratification variables and MNM.

Figure S2. Net classification improvement (NRI) and integrated discrimination improvement (IDI) were compared between the two models. (A) Comparison of two models in the development cohort. (B) Comparison of the two models in the validation cohort.

Table S1. Univariate regression analysis of predictive factors for unfavorable prognosis.

